ON THE ANTIFERMENTATIVE ACTION OF ANTIPLAGUE SERA

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ON THE ANTIFERMENTATIVE ACTION OF ANTIPLAGUE SERA

/Following is the translation of an article by I. V. Domaradskiy, Z. I. Vasil'yeva and G. P. Aparin in the Russian-language periodical Izvestiya Irkutskogo gosudarstvennogo nauchnoissledovatel'skogo protivochumnogo instituta Sibiri i Dal'nego Vostoka (Journal of the Irkutsk State Scientific-Research Antiplague Institute of Siberia and the Far East), Vol 21, 1959, pages 122-127./

Many works devoted to the effect of the sera of immunized and normal animals upon extremely varied vital activities of pathogenic microorganisms have been published. However, very little research has been done on the anti-enzymatic effect of sera to the present time. Nevertheless, investigation of the antigenic properties of bacterial enzymes and the effect of sera on their activity (independent of the mechanism which lies at the base of this effect) is of very great theoretical interest.

If it is postulated that any given enzyme of a pathogenic bacillus has a toxic effect on the host organism by virtue of disintegration of its metabolism, then one of the forms of protection of the affected organism is the development of suitable antienzymes. These would act toward reduction of the activity of the enzyme-toxins ("neutralization"). To this must be added also the therapeutic effect of immune sera.

However, the role of the enzyme as a pathogenetic factor may not be judged solely on the basis of the existence or lack of its antigenic properties. Some enzymes have all the properties of antigens but the corresponding antisera have no effect on them. On the other hand, cases are known in which the antienzymatic action of immune (and to a greater extent, normal) sera is not connected with the existence of antibodies in them.

At the present time we do not know whether plague bacillus has an enzyme which may be attributed participation in the development of the infection process. It is true that one of these enzymes may be

hyaluronidase, the presence of which was postulated by Korobkova. Unfortunately, the investigations conducted in our laboratory did not support the data of the above author. Not one of twenty strains of plague bacillus had the ability to split hyaluronic acid. It may be proposed that the results of Korobkova's investigation may derive from errors of method.

Taking the above into consideration, it was decided to begin the present work with a study of the effect of sera on the activity of one enzyme of oxidation and reduction (catalase) and of a hydrolase (phosphatase).

Being unable to immunize animals with purified preparations of these two enzymes, we used sera of horses immunized with live cultures of plague bacillus /see Note/.

(/Note/ The serum was obtained from the horse "V'yun" (Groundling) before immunization, and following eight cycles of immunization. In tests with guinea pigs innoculated subcutaneously with 10 Dlm plague bacillus, the immune serum exhibited good therapeutic properties (60 percent of the animals survived).

The bacteria were cultured on Chottinger agar with 5 percent serum. The serum was added to the fused agar after cooling to 45 degrees Centigrade. Bacilli obtained from corresponding medium without serum served as control.

The activity of the catalase was determined by the Rokenmacher procedure and was expressed in milligrams of hydrogen peroxide destroyed by the bacillus in 15 minutes while the culture was agitated at room temperature /see Note/.

(/Note/ The test consisted of the following: 5 ml suspension of one-day culture of bacteria, which had been washed three times, containing $1.29 \cdot 10^9$ microbe bodies + 25 ml 0.48 N H_2O_2 solution in 0.03 N phosphate buffer of pH 6.8. The quantity of unconverted hydrogen peroxide was determined in 5 ml fluid by titration with 0.1 N solution of $KMnO_4$ in the presence of 3 ml 7 N H_2SO_4.)

TABLE 1. ACTIVITY OF PLAGUE BACILLUS CATALASE FOLLOWING SINGLE IMPLANTATION ON A MEDIUM CONTAINING SERA

	Media		
	Without Serum (Control)	With Normal Serum	With Immune Serum
Strain YeV	20.4±3.1	16.2±6.1	15.0±4.5
	m = ±0.7	m = ±1.0	m = ±1.0
	V = 15.2%	V = 37.8%	V = 30.0%
Strain 17	19.7±3.8	16.5±4.5	17.2±5.6
	m = ±1.0	m = ±1.2	m = ±1.6
	V = 19.3%	V = 27.3%	V = 32.6%
Strain 1435 (Virulent)	13.0±3.0	12.8±2.6	11.1±2.1
	m = ±1.1	m = ±1.0	m = ±0.8
	V = 23.0%	V = 20.3%	V = 18.9%

Note: m = Average error; V = Coefficient of variation.

As may be seen from Table 1, single cultivation of plague bacillus on media with normal and immune sera has no significant effect on the activity of the catalase of the washed cells. We may speak of a degree of influence of the sera only in the event that the difference in the average magnitude of catalase activity of the strains cultured on media with, and without serum, differs 2.5- to 3-fold from the average error /see Note/. In the experiments with avirulent strain 17 and virulent strain 1435 the ratio of the difference of average magnitudes of catalase activity in cultures obtained from media with and without serum to that of the average error was not less than twofold. In only one series of experiments, in that involving the YeV strain, the difference between the average magnitudes of catalase activity of the culture with and without serum exceeded the average error difference by approximately 2.7-fold. In this case, however, the differences between the normal and immune sera with respect to their ability to suppress the catalase activity could not be established.

(/Note/ Computation was according to the formula $\frac{M_1 - M_2}{m^2_1 + m^2_2}$,

where M_1 and M_2 are the average values of the compared indicators, and $m^2_1 + m^2_2$ are the corresponding average error values.)

TABLE 2. ACTIVITY OF PLAGUE BACILLUS CATALASE FOLLOWING TEN-FOLD TRANSPLANTATION ON MEDIA CONTAINING SERA

	Media		
	Without Serum (Control)	With Normal Serum	With Immune Serum
Strain YeV	27.3±1.9	17.3±2.5	14.2±2.8
	m = ±0.9	m = ±1.1	m = ±1.3
	V = 6.9%	V = 14.8%	V = 19.7%
Strain 17	23.9±3.0	18.2±2.1	16.8±3.5
	m = ±1.4	m = ±1.4	m = ±1.6
	V = 12.5%	V = 17.0%	V = 20.8%

The results of the determination of the catalase activity of two avirulent strains of plague bacillus following ten transplantations on media with-, and without sera (transplantation after a period of one day) are shown in Table 2.

On the basis of these data it follows that extended cultivation of plague bacillus in the presence of sera is accompanied by visible suppression of enzyme activity. At first glance the latter is expressed somewhat more clearly in the case of immune sera, although detailed analysis indicates the lack of substantial difference in the activity of cultures obtained from media with immune, and normal sera (as in the preceeding experiment with YeV strains; cf. Table 1).

Apparently the sera have a non-specific effect on the activity of the catalase of avirulent strains in plague bacillus, and is not connected with the presence of therapeutic and preventive substances in the sera.

The ten-fold transplantation to media with sera is not reflected in the activity of the catalase of virulent strains in plague bacillus. The small effect of sera added to the nutrient media, upon the catalase activity of the virulent strain also is indicated by the coefficient of variation. As is seen from Table 1, the difference in the degree of dispersion of variation orders in the case of the virulent strain is less than in the avirulent strains.

The activity of the phosphatases was determined according to the method of Sagar and his associates. The results were expressed in micrograms of inorganic phosphate for the entire test /see Note/.

(/Note/ Composition of the test: 1 ml washed suspension of cells of a two-day culture of bacteria containing $2 \cdot 10^{10}$ microbe bodies + 2.5 ml 0.5 percent solution of beta-sodium glycerophosphate in veronal buffer of pH 8.0. Incubation was 1 hour at 37°C. Proteins were precipitated by 0.5 ml 30-percent trichloracetic acid. The phosphorus was determined by the Fiske-Subarow method.)

Phosphatase activity was investigated in three strains of plague bacillus (1, 17 and YeV), and in one pseudotubercular strain /see Note/. The latter was used because of the similarity of its antigen structure to that of the plague bacillus. We were the first to establish the presence of phosphatase in the pseudotuberculosis microbe.

(/Note/ Because we used both glycerine-positive (17) and glycerine-negative (1 and YeV) strains of plague bacillus it may be proposed that the intensity of their splitting of glycerophosphate was not connected with the mechanism of glycerine fermentation.)

The data of Table 3 indicate that the presence of sera in the medium in general has no effect on the activity of the phosphatase of the plague and pseudotuberculosis causative agents. In almost all cases the difference between the average values of phosphatase activity of cultures grown on media with, and without sera did not exceed the average error by more than twofold. An exception was strain 1, the phosphatase activity of which decreased slightly following cultivation of the microbe on media containing sera. However, the change in activity of the enzyme is so slight that it is hardly possible to draw a conclusion on this basis. This position is supported by the fact that upon subsequent transplantations to media containing serum, all microbes split a uniform amount of inorganic phosphorus off the beta-glycerophosphate, irrespective of the medium on which they were cultured (Table 4).

Further experiments were based on the "neutralization" type of reaction. In this series washed suspensions of bacteria which had been cultured on media lacking serum were mixed with various dilutions of serum (1:10 to 1:500) and were heated at 37°C for one, or three hours. The control experiments included cell suspensions with the corresponding buffer solution. Next, one of several substrates was added to each test. The destruction of hydrogen peroxide during the investigation of catalase activity, and the accumulation of inorganic phosphate in the course of determination of phosphatase activity were determined following repeated one-hour incubation periods.

TABLE 3. ACTIVITY OF PLAGUE AND PSEUDOTUBERCULOSIS BACILLI PHOSPHATES FOLLOWING SINGLE IMPLANTATION ON MEDIUM CONTAINING SERUM

	Media		
	Without Serum (Control	With Normal Serum	With Immune Serum
Plague Bacillus	29.7±8.2	27.5±9.6	26.7±8.8
Strain YeV	m = ±2.6	m = ±3.0	m = ±2.7
	V = 27.8%	V = 35.0%	V = 32.8%
Strain 1	84.2±9.4	72.3±10.3	70.4±10.0
	m = ±3.1	m = ±3.4	m = ±3.3
	V = 11.1%	V = 14.2%	V = 14.2%
Strain 17	140.5±34.2	125.9±34.5	114.0±29.6
	m = ±10.7	m = ±10.8	m = ±9.2
	V = 24.3%	V = 26.8%	V = 25.9%
Pseudotuberculosis Bacillus, Strain 6	4.7±16.1	40.0±14.9	32.4±9.4
	m = ±6.0	m = ±5.4	m = ±0.5
	V = 36.0%	V = 37.3%	V = 29.0%

TABLE 4. ACTIVITY OF PLAGUE AND PSEUDOTUBERCULOSIS BACILLUS PHOSPHATASE FOLLOWING TEN-FOLD TRANSPLANTATION TO MEDIA WITH SERA

	Media		
	Without Serum (Control)	With Normal Serum	With Immune Serum
Plague Bacillus Strain YeV	37.0±13.8	31.2±10.0	32.4±10.5
	m = ±4.6	m = ±3.3	m = ±3.5
	V = 37.4%	V = 32.2%	V = 32.8%
Strain 1	86.3±7.4	89.1±7.5	87.5±5.7
	m = ±2.4	m = ±2.5	m = ±1.9
	V = 8.6%	V = 8.4%	V = 6.5%
Strain 17	97.6±14.4	94.8±20.7	104.1±18.8
	m = ±4.8	m = ±6.9	m = ±6.3
	V = 14.8%	V = 22.0%	V = 18.1%
Pseudotuberculosis Basillus, Strain 6	43.8±8.0	51.4±19.7	52.9±18.8
	m = ±3.0	m = ±6.5	m = ±6.3
	V = 16.6%	V = 38.6%	V = 35.6%

In view of the homogeneity of the results we obtained, we include only some of the data, for the purpose of illustration of the antienzyme action of the sera tested. From Tables 5 and 6 it may be seen that immune and normal sera do not affect the enzyme activity of washed cells of plague bacillus.

TABLE 5. CATALASE ACTIVITY OF CELLULAR SUSPENSIONS OF STRAIN YeV OF PLAGUE BACILLUS, PROCESSED WITH SERA (1:500)

Time of Incubation of Bacillus (Before Addition of Substrate)	Without Serum (Control)	With Normal Serum	With Immune Serum
1 Hour	23.7	22.4	22.8
3 Hours	20.8	22.4	22.4

TABLE 6. PHOSPHATASE ACTIVITY OF CELLULAR SUSPENSIONS OF STRAIN 17 PLAGUE BACILLUS, PROCESSED WITH SERA (1:50)

Time of Incubation of Bacillus (Before Addition of Substrate)	Without Serum (Control)	With Normal Serum	With Immune Serum
1 Hour	42.1	39.5	41.9
3 Hours	37.4	36.7	40.3

Note: Average data of four determinations.

CONCLUSIONS

Single implantation on media with normal and immune horse sera does not reflect in the activity of the catalase and phosphatase of plague bacillus.

Extensive cultivation of avirulent strains of plague bacillus in the presence of sera lowers the activity of only one enzyme, catalase; the activity of the catalase of virulent strains remains unchanged under these conditions. The influence of the sera on the activity of catalase of avirulent strains of plague bacilli is nonspecific, and is not connected with the presence of therapeutic and preventive substances in them.

Immune and normal sera do not have an antienzyme effect in relation to catalase and phosphatase of washed cells of plague bacillus cultured on media lacking serum.

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